ACUTE AND SUB CHRONIC TOXICITY ASSESSMENT OF DONG TRUNG HA THAO SAPA CAPSULES IN EXPERIMENTAL ANIMALS

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"Dong trung ha thao Sapa" capsules prepared from Cordyceps militaris powder are intended to use for male hypogonadism treatment. So far, the safety of this product has not yet been reported. This study is to evaluate the acute and sub chronic oral toxicity of "Dong trung ha thao Sapa" capsules in experimental animals. The acute toxicity evaluation was carried out according to Litchfield Wilcoxon's method in Swiss mice. Mice were administered orally with increasing doses in the same volume to determine the lowest dose causing 100% death in mice and the highest dose causing no death in mice. The sub-chronic toxicity was evaluated according to WHO and OECD guidelines in Wistar rats with oral doses of 252 mg/kg b.w/day and 756 mg/kg b.w/day in 90 consecutive days. In the course of the acute toxicity test, mice showed no abnormal signs or death. At two doses 252 mg/kg b.w/day and 756 mg/kg b.w/day, the sub chronic toxicity study did not change the rats' body weight, hematological, biochemical parameters, and microscopic of the livers and kidneys during the study period. In conclusion, "Dong trung ha thao Sapa" capsules do not appear to produce acute toxicity in mice and sub chronic toxicity in rats.

Keywords: "Dong trung ha thao Sapa" capsules, Cordyceps militaris, acute toxicity, subchronic toxicity, mice, rats.

I. INTRODUCTION

Nature has been a source of medicinal agents from ancient times and medicinal plants, especially, have formed the basis of the wide variety of traditional medicines used in various countries worldwide.[1] The exclusive use of herbal products for the management of a variety of ailments continues due to easy access, better compatibility, and for economic reasons. "Dong trung ha thao Sapa" capsules are prepared from natural materials, including *Cordyceps militaris* powder combined with several excipients. The capsules intended use is to treat man hypogonadism. So far, there have been no report available on the toxicity of the product. Therefore, in this study, we aimed to evaluate the acute and sub chronic toxicity of "Dong trung ha thao Sapa" capsules in experimental animals.

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II. SUBJECTS AND METHODS

1. The preparation of "Dong trung ha thao Sapa" capsules

"Dong trung ha thao Sapa" capsules were provided by Traphaco Sapa Company, formulated as hard capsules, and each capsule contained 350 mg *Cordyceps militaris* powder

combined with several excipients including PVP, Amidon, and stearate.

- Use: 3 capsules each time, twice a day.
- Storage: Keep in closed packaging, cool and dry place, avoid direct sunlight, not to exceed 30 degrees Celsius.

2. Experimental animals

Wistar rats (weighed 200 - 220 g) and *Swiss* mice (weighed 18 - 22 g) were used in this study. The animals were housed in cages (groups of ten rats or mice/cage) in a room with access to a standard certified rodent diet and water *ad libitum*. They were acclimated to housing for one week before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University

3. Acute toxicity study

Acute toxicity study was carried out according to Litchfield – Wilcoxon.[2]

Groups of mice (10 per group) were fasted for 12h and orally administered "Dong trung ha thao Sapa" capsules at ascending doses that mice could be tolerated. The general symptoms of toxicity and mortality in each group, within 24 hours, were recorded. The median lethal dose (LD50) was estimated by the Litchfield Wilcoxon method. Animals that survived after 24 hours were further observed for 7 days for signs of delayed toxicity.

4. Subchronic toxicity study

Subchronic toxicity studies were carried out according to WHO Guidance and OECD guidelines.[3]

The study was carried out in a continuous 90-day period. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control) was served as the distilled water control at the volume of 10 ml/kg b.w/day by the oral route of administration;
- Group 2 was administered "Dong trung ha thao Sapa" capsules at the dose of 252 mg/kg b.w/day
- Group 3 was administered "Dong trung ha thao Sapa" capsules at the dose of 756 mg/kg b.w/day as the high-dose group.

Animals were treated daily by the oral administration route once a day in the morning for 90 days and were observed daily to detect toxicity signs.

The signs and indexes were checked during the study, including:

- General condition consists of mortality and clinical signs.
- Bodyweight changes.
- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).
- Serum biochemistry: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

The parameters were examined at intervals: before treatment, 30 days, 60 days, and 90 days after treatment. At the end of the experiment, all animals were subjected to a full gross necropsy. Liver and kidney of 30% of rats of each group were removed for histopathological examinations.

5. Statistical analysis

Data were analyzed by the T-test using Microsoft Excel software version 2010. Data were presented as a mean ± standard deviation. A p-value of less than 0.05 is statically significant.

III. RESULTS

1. Acute toxicity study

Table 1. Acute toxicity study of "Dong trung ha thao Sapa" capsules

Group	n	Dose (ml/kg b.w)	Dose (g/kg b.w)	The proportion of deaths (%)	Other abnormal signs
Group 1	10	30	18	0	No
Group 2	10	45	27	0	No
Group 3	10	60	36	0	No
Group 4	10	75	45	0	No

In the oral acute toxicity study, "Dong trung ha thao Sapa" capsules treated animals showed nomortality when observing up to the highest dose level (15.75 g/kg b.w) within 24 h and for an additional 7 days. Also, the animals did not show any sign of acute toxicity such as piloerection, lacrimation, or changes in locomotion and respiration.

2. Sub chronic toxicity study

2.1. General condition

Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes when compared with the control group.

2.2. Body weight change

Table 2. Effect of "Dong trung ha thao Sapa" capsules on body weight change

Time	Bodyweight (gram)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	180.00 ± 11.06	180.50 ± 13.43	179.00 ± 13.90	> 0.05
30 days after treatment	197.50 ± 18.45	199.50 ± 13.83	201.00 ± 20.79	> 0.05
p (before - after)	< 0.05	< 0.05	< 0.05	
60 days after treatment	213.50 ± 25.83	214.50 ± 12.57	217.50 ± 25.52	> 0.05
p (before - after)	< 0.05	< 0.05	< 0.05	
90 days after treatment	225.00 ± 20.74	227.00 ± 12.95	230.00 ± 20.95	> 0.05
p (before - after)	< 0.05	< 0.05	< 0.05	

Table 2 showed that no significant difference was observed between groups treated "Dong trung ha thao Sapa" capsules and control group – (group 1) ($p > 0.05$).

2.3. Hematological parameters

Table 3. Effect of "Dong trung ha thao Sapa" capsules on hematopoietic function

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
Red blood cells count (T/L)	Group 1	9.07 ± 0.86	8.97 ± 0.82	8.95 ± 0.83	8.85 ± 0.90
	Group 2	8.86 ± 0.62	8.72 ± 0.70	8.59 ± 1.01	8.47 ± 0.73
	Group 3	9.19 ± 0.74	9.08 ± 0.99	8.90 ± 0.79	8.62 ± 0.71
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hemoglobin level (g/dL)	Group 1	12.78 ± 1.25	12.81 ± 1.10	12.69 ± 1.28	12.58 ± 0.78
	Group 2	12.82 ± 1.08	12.74 ± 1.06	12.63 ± 0.94	12.29 ± 1.13
	Group 3	13.02 ± 0.96	12.94 ± 1.28	12.53 ± 0.99	12.57 ± 0.89
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Group 1	48.68 ± 4.74	48.32 ± 3.17	48.40 ± 2.75	48.33 ± 2.21
	Group 2	49.72 ± 4.13	48.51 ± 3.13	48.29 ± 2.50	48.03 ± 1.93
	Group 3	49.77 ± 4.18	48.92 ± 2.79	48.79 ± 3.75	48.74 ± 3.53
	p	> 0.05	> 0.05	> 0.05	> 0.05
MCV (fl)	Group 1	53.40 ± 2.22	52.50 ± 2.72	52.90 ± 1.66	52.30 ± 2.54
	Group 2	53.10 ± 2.64	52.30 ± 2.31	52.60 ± 1.96	52.10 ± 1.85
	Group 3	53.50 ± 2.07	52.70 ± 1.77	52.40 ± 1.65	52.50 ± 1.08
	p	> 0.05	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Group 1	679.90 ± 98.63	704.50 ± 88.00	674.30 ± 76.33	719.10 ± 84.46
	Group 2	670.50 ± 87.04	711.40 ± 95.71	714.80 ± 78.15	732.70 ± 74.49
	Group 3	654.40 ± 94.62	663.60 ± 86.38	684.30 ± 98.01	665.20 ± 66.84
	p	> 0.05	> 0.05	> 0.05	> 0.05

Table 4. Effects of "Dong trung ha thao Sapa" capsules on total WBC count and WBC differentials

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
Red blood cells count (T/L)	Group 1	9.07 ± 0.86	8.97 ± 0.82	8.95 ± 0.83	8.85 ± 0.90
	Group 2	8.86 ± 0.62	8.72 ± 0.70	8.59 ± 1.01	8.47 ± 0.73
	Group 3	9.19 ± 0.74	9.08 ± 0.99	8.90 ± 0.79	8.62 ± 0.71
	p	> 0.05	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
Hemoglobin level (g/dL)	Group 1	12.78 ± 1.25	12.81 ± 1.10	12.69 ± 1.28	12.58 ± 0.78
	Group 2	12.82 ± 1.08	12.74 ± 1.06	12.63 ± 0.94	12.29 ± 1.13
	Group 3	13.02 ± 0.96	12.94 ± 1.28	12.53 ± 0.99	12.57 ± 0.89
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Group 1	48.68 ± 4.74	48.32 ± 3.17	48.40 ± 2.75	48.33 ± 2.21
	Group 2	49.72 ± 4.13	48.51 ± 3.13	48.29 ± 2.50	48.03 ± 1.93
	Group 3	49.77 ± 4.18	48.92 ± 2.79	48.79 ± 3.75	48.74 ± 3.53
	p	> 0.05	> 0.05	> 0.05	> 0.05
MCV (fl)	Group 1	53.40 ± 2.22	52.50 ± 2.72	52.90 ± 1.66	52.30 ± 2.54
	Group 2	53.10 ± 2.64	52.30 ± 2.31	52.60 ± 1.96	52.10 ± 1.85
	Group 3	53.50 ± 2.07	52.70 ± 1.77	52.40 ± 1.65	52.50 ± 1.08
	p	> 0.05	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Group 1	679.9 ± 98.63	704.5 ± 88.00	674.3 ± 76.33	719.1 ± 84.46
	Group 2	670.5 ± 87.04	711.4 ± 95.71	714.8 ± 78.15	732.7 ± 74.49
	Group 3	654.4 ± 94.62	663.6 ± 86.38	684.3 ± 98.01	665.2 ± 66.84
	p	> 0.05	> 0.05	> 0.05	> 0.05

As shown in table 3 and table 4, there was no significant difference in red blood cells count, hematocrit, hemoglobin level, MCV, platelet count, total WBC count, and WBC differentials between groups treated with “Dong trung ha thao Sapa” capsules and group 1 (p > 0.05).

2.4. Liver parameters

Table 5. Effects of “Dong trung ha thao Sapa” capsules on liver parameters

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
AST level (UI/L)	Group 1	83.70 ± 14,74	85.40 ± 10,99	80.70 ± 11,24	79.60 ± 10,22
	Group 2	89.70 ± 17,52	94.50 ± 12,73	90.10± 13,08	87.20 ± 11.90
	Group 3	87.40 ± 15,88	94.80 ± 17,24	90.60 ± 13.85	85.90 ± 11.47
	p	> 0.05	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
ALT level (UI/L)	Group 1	41.70 ± 9.65	43.50 ± 9.16	41.90 ± 8.50	40.70 ± 6.58
	Group 2	42.40 ± 8.38	44.30 ± 9.66	44.20 ± 9.82	43.40 ± 9.19
	Group 3	42,60 ± 8.64	45.20 ± 9.55	44.80 ± 7.41	42.10 ± 6.08
	p	> 0.05	> 0.05	> 0.05	> 0.05
Total bilirubin (mmol/L)	Group 1	13.50 ± 0,37	13.36 ± 0.38	13.39 ± 0.42	13.49 ± 0.48
	Group 2	13.48 ± 0.44	13.41 ± 0.22	13.37 ± 0.35	13.46 ± 0.55
	Group 3	13.44 ± 0,33	13.40 ± 0.24	13.45 ± 0.41	13.62 ± 0.30
	p	> 0.05	> 0.05	> 0.05	> 0.05
Albumin concentration (g/dL)	Group 1	3.27 ± 0.31	3.25 ± 0.22	3.20 ± 0.33	3.22 ± 0.19
	Group 2	3.28 ± 0.36	3.23 ± 0.29	3.27 ± 0.34	3.25 ± 0.31
	Group 3	3.25 ± 0.27	3.28 ± 0.34	3.29 ± 0.24	3.26 ± 0.25
	p	> 0.05	> 0.05	> 0.05	> 0.05
Total cholesterol concentration (mmol/L)	Group 1	1.55 ± 0.22	1.56 ± 0.18	1.52 ± 0.23	1.57 ± 0.21
	Group 2	1.56 ± 0.21	1.62 ± 0.20	1.53 ± 0.26	1.55 ± 0.25
	Group 3	1.54 ± 0.24	1.59 ± 0.21	1.57 ± 0.27	1.56 ± 0.20
	p	> 0.05	> 0.05	> 0.05	> 0.05

There were no significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT) level, total bilirubin, albumin concentration, and total cholesterol concentration between groups treated with “Dong trung ha thao Sapa” capsules and the control group (p > 0.05). The results are shown in Table 5.

2.5. Kidney function

Table 6. Effects of “Dong trung ha thao Sapa” capsules on serum creatinine level

Days	Creatinine (mg/dl)			p (t- test Student)
	Group 1	Group 2	Group 3	
Before treatment	0.86 ± 0,13	0.89 ± 0,15	0.87 ± 0,16	> 0.05
After 30 days	0.88 ± 0,14	0.86 ± 0,19	0.90 ± 0,17	> 0.05
p (before – after)	> 0,05	> 0.05	> 0.05	
After 60 days	0.83 ± 0.12	0.82 ± 0.15	0.84 ± 0.18	> 0.05
p (before - after)	> 0.05	> 0,05	> 0.05	
After 90 days	0.84 ± 0,16	0.85 ± 0,10	0.83 ± 0,13	> 0.05
p (before – after)	> 0.05	> 0.05	> 0.05	

As shown in table 6, after treatment, “Dong trung ha thao Sapa” capsules caused no significant difference in serum creatinine levels between the control group and 2 treated groups (p > 0.05).

2.6. Histopathological examination

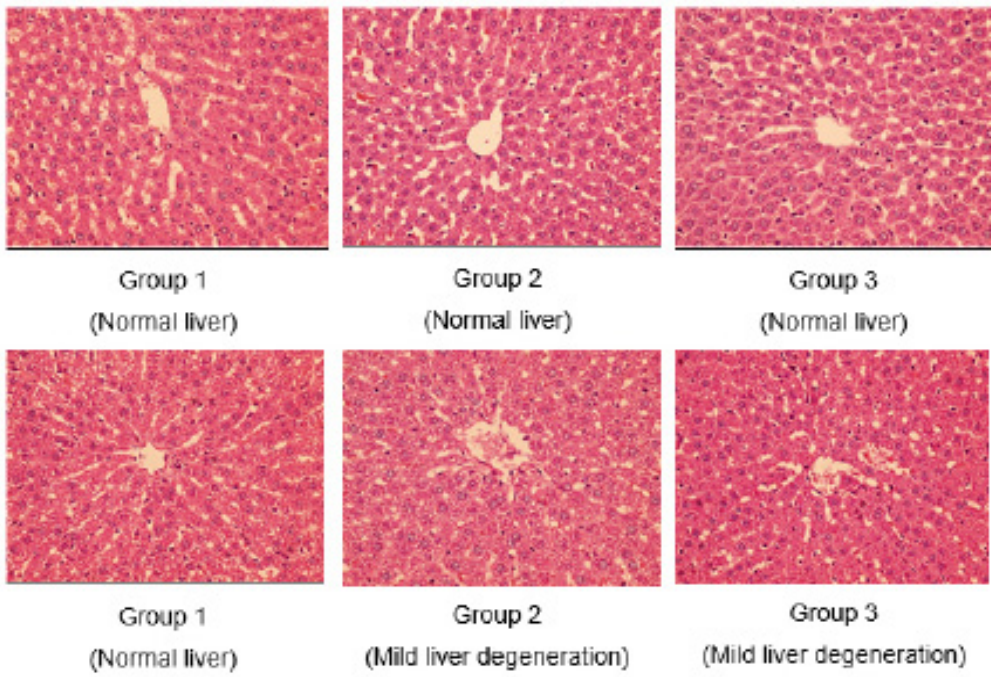

Figure 1. Histopathological images of the liver (HE × 400)

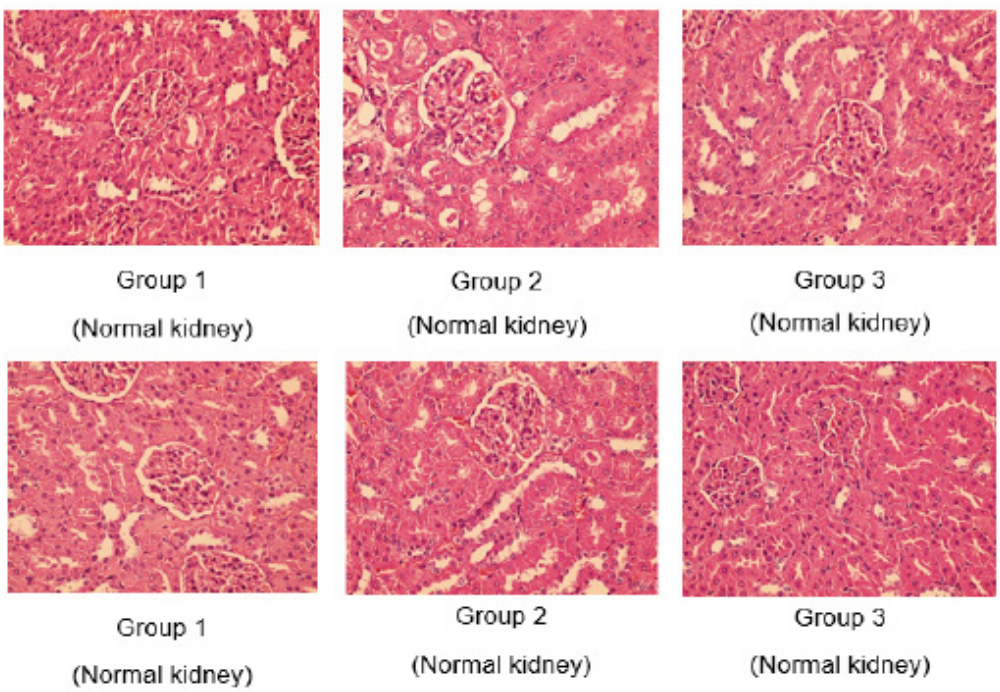

Figure 2. Histopathological images of the kidney (HE × 400)

No gross lesions or changes in size were observed when subjected all experimental rats to a full gross necropsy which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There was no significant difference in histopathological examination of liver and kidney between rats treated with "Dong trung ha thao Sapa" capsules and the control group after 90 days of treatment (Figures 1 and 2).

IV. DISCUSSION

In this experiment, an acute oral toxicity test showed that "Dong trung ha thao Sapa" capsules were tolerated up to 15.75 g/kg b.w (approximately 31.25 times as high as the recommended human dose). Moreover, no signs of toxicity and no mortality were observed for a continuous seven days. As a result, oral LD_{50} of "Dong trung ha thao Sapa" capsules was not determined in mice. As defined by WHO, "Dong trung ha thao Sapa" derived from herbal medicine[4] was a safe product.

Toxicity is the degree to which a substance can harm humans or animals. Toxicity can refer to the effect on a cell (cytotoxicity), an organ (e.g. renal or liver toxicity), or the whole organism. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animal models to predict the toxicity and provide guidelines for selecting 'safe' therapeutic doses in humans. A sub-chronic toxicity study information on the effects of repeated oral exposure can indicate the need for longer-term studies.[5] Sub chronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. Specifically, they provide information on target organ toxicity.[6]

The body weight changes serve as a sensitive indicator of the general health status of animals.[6] Weights were observed in all animals treated with "Dong trung ha thao Sapa" capsules. It can be stated that "*Dong trung ha thao Sapa" capsules* did not interfere with animals' normal metabolism as corroborated by the non-significant difference from animals distilled water control group.

The hematopoietic system is one of the most sensitive targets of toxic compounds and is an essential index of physiological and pathological status in men and animals. Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data is translated from animal studies.[5] After 30 days, 60 days, and 90 days post treatment, there were no significant difference in total red blood cells, hematocrit, hemoglobin level, platelet count, total WBC count and WBC differentials between groups treated with "Dong trung ha thao Sapa" capsules and the control group. So it can be concluded that the administration of "Dong trung ha thao Sapa" capsules did not affect the hematological profile and blood formation process.

Analysis of the kidney and liver is critical in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.[7] The liver releases aspartate aminotransferase (AST), alanine aminotransferase (ALT) and an elevation in plasma concentration is an indicator of liver damage.[5] The non-significant changes in ALT and AST in both male and female rats at all doses indicated that "Dong trung ha thao Sapa" capsules had no deleterious effect on

liver function. Creatinine levels can be used in describing the function of the kidneys.[4] The blood biochemistry level of control and "Dong trung ha thao Sapa" capsules treated rats at two dose levels were presented no significant difference between the treated groups and the control one ($p > 0.05$), so that "Dong trung ha thao Sapa" capsules did not affect the liver and kidney function.

The histopathological examination revealed the alteration in cell structure when viewed under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of "Dong trung ha thao Sapa" capsules. Our study showed no significant difference in histopathological examination of the liver and kidney between groups treated with "Dong trung ha thao Sapa" capsules and the control group.

Overall, there was no significant difference observed in blood profile, biochemistry parameters, and histopathological observations of kidney tissues between groups treated "Dong trung ha thao Sapa" and the control group. However, there were 1/3 samples with evidence of mild degeneration liver cells, therefore further studies of liver cells histopathology are recommended.

V. CONCLUSION

No signs of toxicity and no mortality were observed in mice treated with "Dong trung ha thao Sapa" capsules at a dose of 15.75 g/kg (approximately 31.25 times as high as the recommended human dose). Oral LD50 of "Dong trung ha thao Sapa" capsules were not determined in mice.

For a continuous 90 days, "Dong trung ha thao Sapa" capsules at doses 252 mg/kg b.w/day and 756 mg/kg b.w/day did not produce any toxic signs or evident symptoms of sub chronic toxicity.

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